2004

# Prometaphase Chromosome Preparation from Mouse Spleen (C57Bl/6)

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## Reagents

**Acetic acid, glacial**
**Antibiotic-Antimycotic 100x**
10,000 U/ml Penicillin G sodium, 10,000 µg/ml streptomycin sulfate, 25 µg/ml amphotericin B
Gibco BRL, Cat. 15240-013
**Colcemid, KaryoMAX Colcemid Solution, 10 µg/ml**
Gibco BRL, Cat. 15210-016
**Concanavalin A (5 µg/µl)**
Sigma, Cat. C-5275
**Fetal Bovine Serum (FBS) heat inactivated**
Gibco BRL, 16140-022
**L-Glutamine-200 mM, 100x**
Gibco BRL, 25030-016
**Homogenizer**
Thomas Scientific, Cat. 3431D7
**Lipopolysaccharides (LPS) 5mg**
Sigma, Cat. L-2637
**Methanol**
**Methotrexate, 500 mg**
Sigma, Cat. M 8407
**Potassium chloride (KCl)**
**RPMI Medium 1640**
Gibco BRL, Cat. 21870-050

## Preparation

**Reagents**

| | **Amount** |
|---|---|
| **Concanavalin A** | |
| Concanavalin A | 5 mg |
| Sterile water | 1 ml |

For a stock solution of 5µg/µl

**RPMI Complete Medium**

| **Components** | **Amount** |
|---|---|
| RPMI Medium 1640 | 440 ml |
| Antibiotic-Antimycotic, 100X | 5 ml |
| L-Glutamine-200 mM, 100X | 5 ml |
| Fetal Bovine Serum (FBS) | 50 ml |

**Fixative**
Prepare fresh: methanol/acetic acid (3:1)

**Hypotonic Solution**
0.075M KCl in distilled water

**Lipopolysaccharides (LPS)**

| | |
|---|---|
| Lipopolysaccharides (LPS) | 25 mg |
| Sterile water | 1 ml |

Use 1:1000 dilution for a final concentration of 25 µg/ml of culture

**MTX stock**
Make an initial stock of $10^{-3}$ M in $H_2O$ and then dilute to $10^{-5}$ M

**BrdU stock**
1 mg/ml in distilled water

## Procedure

1. Prepare tissue culture flasks. To one T75 flask, add:

| Components | Amount |
|---|---|
| Prepared media | 20 ml |
| Concanavalin A (5µg/µl) | 30 µl |
| Lipopolysaccharides (LPS) | 25 µl |

2. Isolate spleen from mouse. Transport in RPMI 1640.

3. Place three spleens into a homogenizer with 3 ml of plain RPMI media. Grind well.

4. Transfer 0.5 ml to one T75 flask.

5. Incubate at 37°C for 24 hr. After 24 h add 200 µl of MTX stock ($10^{-5}$M) to 20 ml of culture (MTX final concentration of $10^{-7}$M); mix well and incubate an additional 17 h.

6. After 17 h centrifuge the content of the flasks, remove the supernatant, and wash the pellet twice with plain media.

7. After the second wash resuspend the pellet in 20 ml of RPMI 1640 10% BSA and transfer to a T75 flask.

8. Add 500 µl of the BrdU stock (1mg/ml) to a final concentration of 25 $\mu$g/ml (minimize light exposure).

9. Incubate for 5 h 30 min at 37°C.

10. For the last 10 min of the incubation add 20 µl of Colcemid stock (10 $\mu$g/ml) to
a final concentration of 0.06 $\mu$g/ml .

11. Centrifuge cultures for 10 min.

12. Transfer to 50 ml centrifuge tubes and centrifuge at 1000 rpm for 10 min.

13. Remove supernatant.

14. Gently add 10 ml 0.075M KCl (prewarmed to 37°C) to each tube and resuspend pellet.

15. Incubate tubes at 37°C for 15 min.

16. Following incubation, add a few drops of freshly prepared fixative.

17. Centrifuge at 1000 rpm for 10 min.

18. Remove supernatant.

19. Wash pellet with freshly prepared fixative, at least 3 times.

20. Store pellet under fixative at -20°C until ready to prepare slides.